U. S. DEPARTMENT OF AGRICULTURE

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INOCULATION OF LEGUMES AND NONLEGUMES WITH NITROGEN-FIXING AND OTHER BACTERIA

BY THE CULTIVATION OF LEGUMES large quantities of nitrogen can be obtained from the air, but for all cultivated nonlegumes the nitrogen present in the soil alone is available.

Nitrogen fixation from the air takes place only if the legumes harbor the proper bacteria in their root nodules.

These nodules are usually found on native and commonly cultivated legumes, whereas legumes planted for the first time in a locality remain frequently without nodules because of the absence of their bacteria.

Legumes without well-developed root nodules as a rule do not attain a fully satisfactory development. Artificial inoculation with their specific bacteria is advisable in such cases; but proper tillage, together with the application of lime, phosphate, and potash, deserves equal attention.

For the inoculation of legumes, soil can be used, if it contains the proper bacteria and is free from weed seeds and plant diseases and parasites, or artificial cultures may be obtained from Government or State institutions or from reliable firms.

The inoculation of nonlegumes as well as of the soil itself with various beneficial bacteria has been tried repeatedly, but without success.

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INOCULATION OF LEGUMES AND NON-LEGUMES WITH NITROGEN-FIXING AND OTHER BACTERIA

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INTRODUCTION

THE FIRST EXPERIMENTS in the inoculation of legumes were made in Europe about 40 years ago, and similar tests with nonlegumes followed some 10 years later. In most cases the results of these early experiments were not satisfactory, and it was evident that thorough investigations of the inoculation of legumes were urgently needed. These were begun by the United States Department of Agriculture in 1901, and during the next 10 years so much scientific work along these lines was conducted in America as well as in Europe that every year increasing numbers of cultures for the inoculation of legumes were sent out to farmers and used by them to great advantage. Several investigators in Europe tried to get similar results by the inoculation of nonlegumes, but failed.

Between 1902 and 1913 a number of publications were issued by the United States Department of Agriculture to spread among the farmers accurate knowledge of this new method of increasing the yield of crops at a very moderate cost and also to point out to scientific workers the precautions that must be observed in order to obtain satisfactory results. All these publications are now out of print.

At present several hundred thousand cultures of bacteria for the inoculation of legumes are distributed annually by the United States Department of Agriculture, the State experiment stations, and commercial concerns; but as an estimate indicates that all these cultures are sufficient for only about 2 per cent of the legumes planted in this country, further extension of this work seems very desirable.

From time to time new brands of cultures for the inoculation of nonlegumes are offered by commercial concerns to farmers, despite the fact that no efforts made by scientific workers in this direction have been crowned with success. As a rule, these preparations are sold for legumes as well as for nonlegumes, and reports of the satisfactory results obtained with legumes are frequently used in advertisements as the basis for recommending their purchase for nonlegumes. High-pressure sales methods often help to make such attempts very successful for the manufacturer, though not for the farmer.

To give every farmer an opportunity to gain a clear understanding of the whole situation, the most important facts relating to the subject will be presented in the following pages. If now and then some difficulty arises which has not been anticipated here, an inquiry should be sent to the agricultural experiment station of the State in which the farmer resides or to the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. Impartial information, based upon all facts available, will be given free of charge, and, if necessary, experiments will be conducted to obtain definite data which will aid the farmer in his work.

FIG. 1.—Red-clover root with nodules

LEGUMES AND NONLEGUMES

The custom of dividing all cultivated plants into the two groups of legumes and nonlegumes is well founded. It has been known since ancient times that the so-called legumes—peas, beans, vetches, clovers, alfalfa, lupines, and related plants—enrich the soil, whereas a continuous growth of cereals and other nonlegumes leads to a more or less rapid decline in soil productivity. After a more complete knowledge of plant nutrition had been attained in the course of the last century it was found that this difference is mainly due to the manner in which these two groups of plants obtain their nitrogen. All cultivated legumes are able to take nitrogen from the air; all cultivated nonlegumes must find it in the soil. If a soil is rich in nitrogen, the legumes, too, may draw on this source, though they do not need it. Pure sand, devoid of all nitrogen, permits a healthy growth of legumes if all other conditions for their normal development are present.

Most important among these other conditions is the presence on the seeds or in the soil of certain bacteria that will enable the legumes to make use of atmospheric nitrogen. It is not the legume itself but

these bacteria that gather the free nitrogen and transform it into compounds which are taken up by the host plant. The laboratory where the bacteria perform this wonderful transformation, that no chemist has yet been able to imitate, is in the root nodules visible on healthy legume plants.

FIG. 2.—Alfalfa nodules (*A*); vetch nodules (*B*)

Figures 1 to 3 show these peculiar formations upon the roots of clover, alfalfa, vetch, Lima beans, cowpeas, and soy beans. There are considerable differences in the shape and size of the single nodules. Generally those upon plants introduced more recently from Asia are more spherical and larger than those found upon the legumes that are native in Europe and America. Frequently many small nodules are united in clusters, like those shown in Figures 2 and 4.

The number of nodules which may develop on a plant root varies from a few to several thousand. As many as 5,000 have been counted

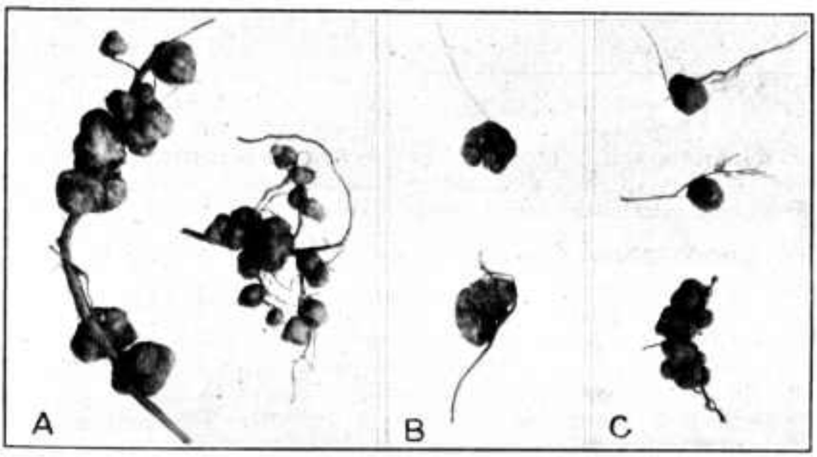

FIG. 3.—Nodules of cowpeas (*A*), soy beans (*B*), Lima beans (*C*)

on individual plants of garden bean plants growing in a greenhouse. It is not to be expected that such large numbers will be found under the conditions existing in extensive fields. The distribution of the nodules on the root is of greater importance, because it shows the distribution of the legume bacteria in the soil. (Fig. 5.)

As with all cultivated legumes, root nodules occur on many wild relatives, which in pastures and meadows play an important rôle. (Fig. 6.) They maintain the productivity of the soil and increase the food value of its product. It is probable that much of the vast quantity of nitrogen stored in our prairie soils has been drawn from the air by the root-nodule bacteria of wild-growing legumes.

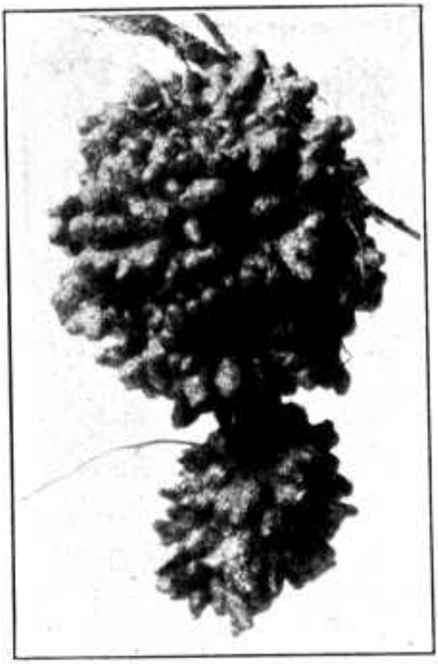
FIG. 4.—Velvet-bean nodules

A few wild-growing legumes, of which the most common are honey locust, Kentucky coffee tree, and wild senna, apparently do not produce nodules; at least, none have been found on them thus far. On the other hand, there are several wild-growing nonlegumes that produce root nodules very similar to those of legumes. The alder tree (Alnus), Jersey tea and mountain balm (Ceanothus), silverberry (Elaeagnus), and Buffalo berry (Lepargyrea), as well as the Cycadaceæ, are such exceptions. (Figs. 7 and 8.) All these plants are of little or no agricultural value. For practical purposes the general statement therefore remains true that the cultivated legumes are able to utilize atmospheric nitrogen with the help of their root-nodule bacteria, whereas cereals, potatoes, beets, and other cultivated nonlegumes are dependent on the nitrogen available in the soil. That nonlegumes may be benefited by nitrogen-fixing bacteria in the soil itself will be discussed presently, but the nonlegumes take little active part in this process.

ROOT-NODULE BACTERIA OF LEGUMINOUS PLANTS

BACTERIA

Like all other bacteria, those in the root nodules of leguminous plants are so very small that powerful microscopes must be used to make them clearly visible. Their form varies to some extent, as may be seen by examining Figures 9 and 10. The real meaning of the thousandfold magnification applied in these cases becomes evident when we consider how a cat, for instance, would look if it could be magnified a thousand times. If its real dimensions are 20 by 10 by 4 inches, they would be increased to approximately 1,650 by 825 by 330 feet. Such a comparison is necessary to give a correct conception of how unbelievably small bacteria really are.

Their minute size makes it easily possible for bacteria to enter the roots of plants and to penetrate the soil to considerable depths. Furthermore, bacteria are able to multiply very rapidly if environ-

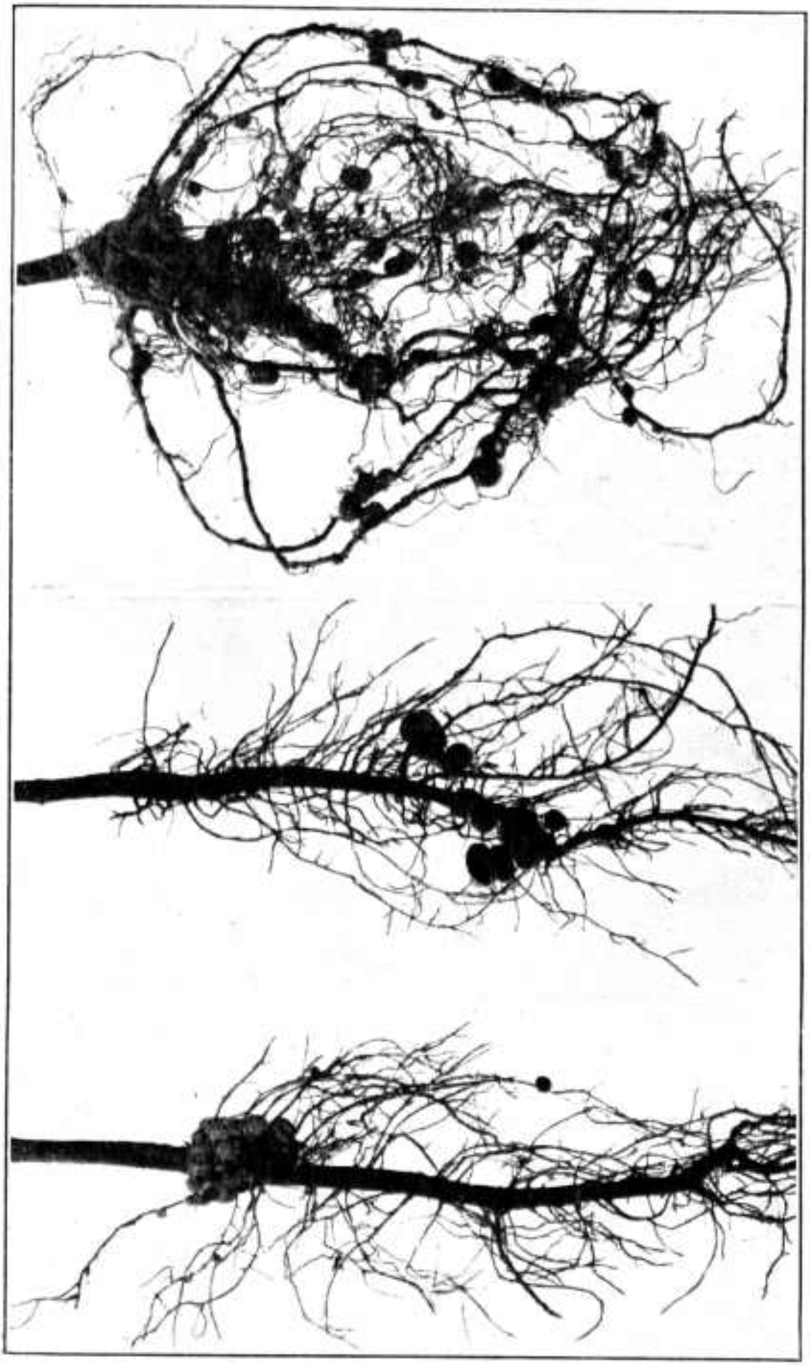

FIG. 5.—Three soy-bean roots, showing the different locations of root nodules

mental conditions are suitable. Within half an hour a bacterium may double its size and then divide into 2 bacteria of original dimensions; 30 minutes later these 2 may make 4; after another hour there will be 16; after the next hour 64; and after about 15 hours the single bacterium may have given rise to not less than 1,000 millions of its kind. Quantities like this become visible to the naked eye as very small slime droplets, usually of a whitish appearance. In the laboratories bacteria are grown in liquids, or upon jellies, or directly in soil. The turbidity and the whitish sediment visible in liquid cultures as well as the slimy layers on jelly cultures always contain very large numbers of active organisms if the cultures are not too old.

FIG. 6.—Roots of a locust tree with many nodules

Despite the minute size of the bacteria, every strain of them has its peculiarities, a higher or lower efficiency, just as with breeds of higher organisms. A very important task in the preparation of cultures consists therefore in the selection and propagation of the most active and efficient strains of bacteria. Although all root-nodule bacteria display many common features, there are certain marks which serve for a more or less accurate differentiation. Most important among these is, of course, the behavior of the bacteria toward leguminous plants. Certain strains of nodule bacteria invade the roots of one kind of legume only, others are much less particular. Numerous tests have shown that for practical purposes the commonly cultivated legumes may be divided into the seven following groups, each of which has its special bacteria.

1.—*Legumes inoculated by alfalfa bacteria*

Common name	Scientific name
Alfalfa	Medicago sativa.
Bitter clover	Melilotus indica.
Button clover	Medicago orbicularis.
California bur clover	Medicago denticulata.
Fenugreek	Trigonella foenumgraecum.
Southern bur clover	Medicago maculata.
White sweet clover	Melilotus alba.
Yellow sweet clover	Melilotus officinalis.
Yellow trefoil	Medicago lupulina.

West of the Mississippi River soils are usually found stocked with alfalfa bacteria, and the spread of sweet clover along the railroad rights of way and the roadsides as well as the planting of alfalfa and sweet clover by farmers has tended to give wide distribution to

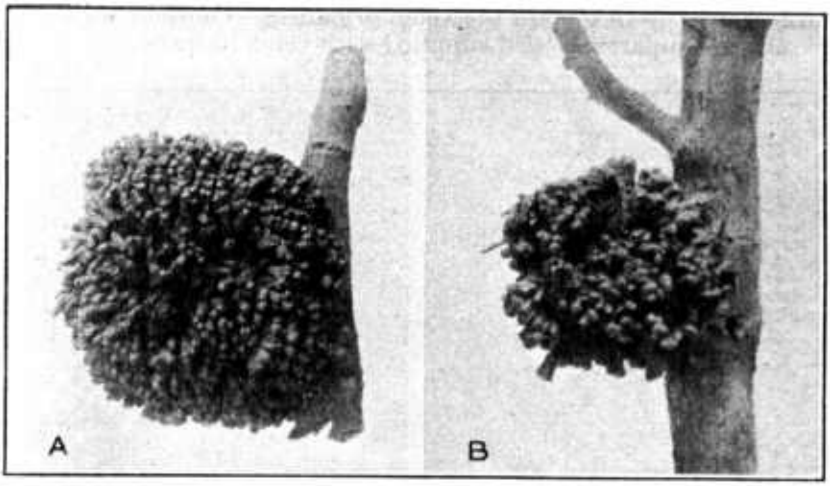

FIG. 7.—Alder nodules (*A*); Jersey-tea nodules (*B*)

the alfalfa strain of nodule organism in the eastern part of the United States.

2.—*Legumes inoculated by red-clover bacteria*

Common name	Scientific name
Alsike clover	Trifolium hybridum.
Crimson clover	Trifolium incarnatum.
Hop clover	Trifolium agrarium.
Low hop clover	Trifolium dubium.
Mammoth red clover	Trifolium pratense perenne.
Rabbit-foot clover	Trifolium arvense.
Red clover	Trifolium pratense.
White clover	Trifolium repens.

Red and other clovers of the genus Trifolium have long been standard legume crops in the northeastern part of the United States, which accounts for the fact that in practically all of the soils in that section nodules occur naturally on these plants. This is true also of the southeastern section of the United States, where crimson clover has long been used as a green-manure crop, occupying the land during the winter.

3.—*Legumes inoculated by vetch bacteria*

Common name	Scientific name
Canada field pea	Pisum sativum arvense.
Common vetch	Vicia sativa.
Garden pea	Pisum sativum.
Hairy vetch	Vicia villosa.
Broad beau (horse bean)	Vicia faba.
Lentil	Lens esculenta.
Narrow-leaf vetch	Vicia angustifolia.
Purple vetch	Vicia atropurpurea.
Sweet pea	Lathyrus odoratus.

The vetch strain of nodule bacteria is present in many localities, because of the presence of wild vetches and the long-continued practice of growing garden peas in the family garden and crop vetches in the field. There are many soils in the Middle West and East, however, that seem to need the vetch organism. On the Pacific coast the soils are apparently well supplied with vetch bacteria.

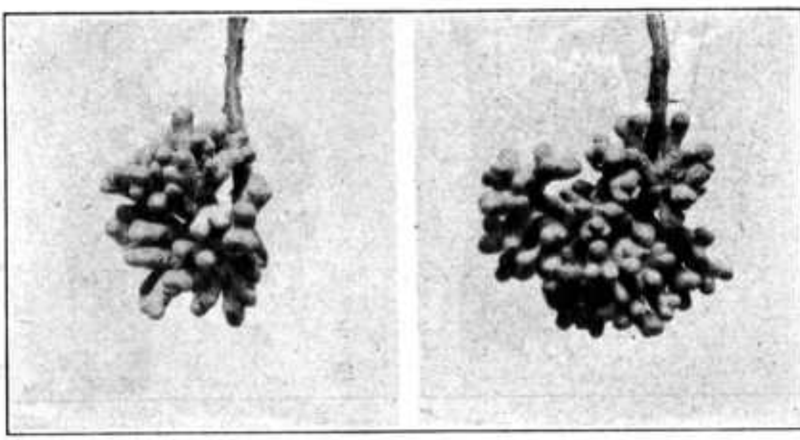

FIG. 8.—Cycas nodules

4.—*Garden and navy-bean bacteria*

The bacteria of common garden beans and navy beans are interchangeable. They are not related to any of the strains previously mentioned. Their distribution is very wide, on account of the almost universal practice of planting some variety of garden bean.

5.—*Lupine bacteria*

Lupine bacteria are rare in all soils where their host plants have not been grown before. Accordingly, the following plants, as a rule, are benefited by inoculation:

Common name	Scientific name
Blue lupine	Lupinus angustifolius.
Serradella	Ornithopus sativus.
Sundial (wild) lupine	Lupinus perennis.
European yellow lupine	Lupiuus luteus.

6.—*Legumes inoculated by cowpea bacteria*

Common name	Scientific name
Cowpea	Vigna sinensis.
Florida beggarweed	Desmodium purpureum.
Jack bean	Canavalia ensiformis.
Japan clover	Lespedeza striata.
Kudzu	Pueraria thunbergiana.
Lima bean	Phaseolus lunatus macrocarpus.
Partridge pea	Chamaecrista fasciculata.
Peanut	Arachis hypogaea.
Pigeon-pea	Cajanus indicus.
Tick trefoil	Desmodium canescens.
Tepary bean	Phaseolus acutifolius.
Deering velvet bean	Stizolobium deeringianum.

The cowpea has been the universal summer legume crop of the South; hence the bacteria of this legume are widely distributed in that section. The fact that many of the native wild legumes

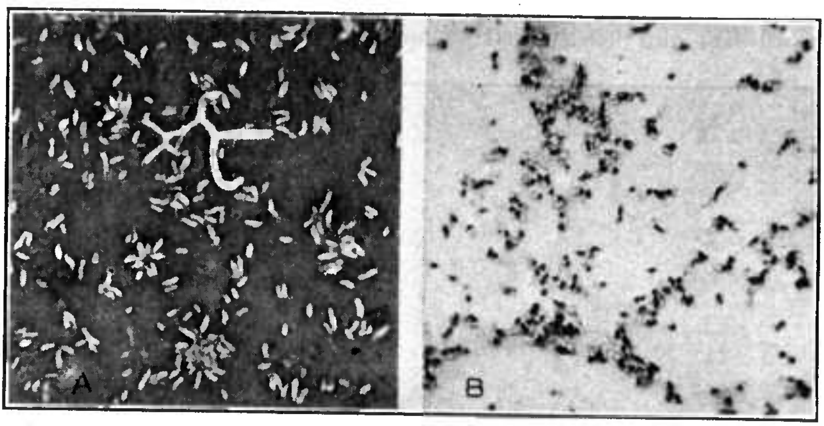

FIG. 9.—Vetch bacteria (*A*) unstained, cowpea bacteria (*B*) stained. × 1,000

and some of the other cultivated legume crops of the South have similar bacteria in their root nodules also contributes to their extensive natural distribution.

7.—*Soy-bean bacteria*

The soy bean, unlike any of the foregoing crops, is associated with bacteria not related to any of the commonly known strains. This, together with the fact that soy beans have been grown extensively for only 25 years in this country, indicates the necessity for artificial inoculation when planted for the first time. In many sections of the South, however, soy beans require no inoculation, since they have been grown there for some time.

NODULES

When a plant is examined for its nodules these will be found either evenly distributed over all roots or most of them close together in a small area near the crown. (Fig. 5.) Only young roots

are entered by the bacteria. If these are brought into the soil with inoculated seed, the infection will take place very early, close to the crown. On the other hand, if only those bacteria that are widely scattered in the soil become active, the nodules will appear later on the lateral roots. The earlier the nodules are formed the more nitrogen will be fixed, as a rule.

All legume root nodules are annual growths, whether they occur on perennial or annual legumes. On account of their tender nature, nodules are easily rotted away when the plant begins to decline or assumes a dormant state. By this means the bacteria are returned to the soil in much-increased numbers. It is sometimes difficult to find nodules on an alfalfa plant after two or three years' growth when the taproot is firmly established and conditions are not favorable for the development of surface roots.

EELWORMS

Other organisms, too, may penetrate the legume roots and cause deformities and swellings that may be mistaken for normal root

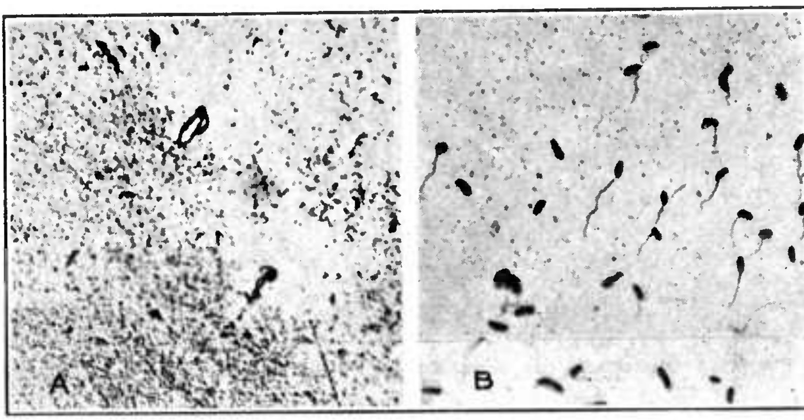

FIG. 10.—Vetch bacteria (*A*) with flagella stained, soy-bean bacteria (*B*) with flagella stained. × 1,000

nodules. Nematodes, or eelworms, may form such galls, and the crown-gall bacterium, which produces tumors on many other plants, may also infect the roots of a legume, causing swellings, which should not be confounded with normal root nodules. Figure 11 shows such abnormal formations. As a rule, plants infected by these organisms do not look healthy. In case of doubt, such material may be sent for examination to a State agricultural experiment station or to the United States Department of Agriculture.

INSECTS

Root-eating insects also by their destruction of nodules play a part in preventing the fixation of nitrogen. One that is widely known at the present time, the bean leaf beetle,[1] is noticeably destructive, mainly in the Southern States. This beetle eats the leaves of some of the

[1] *Ceratoma trifurcata* Forst.

Fig. 11.—Cowpea roots showing nematode galls (*A*) and normal nodules (*B*)

common legumes, such as the garden bean, cowpea, and beggarweed, and lays its eggs at the base of a plant. When the larvæ appear, they feed on the roots and nodules of the plant. (Fig. 12.) Experiments made in Mississippi indicate that nitrogen fixation through the agency of nodule bacteria may be practically stopped by the ravages of these larvæ. To a certain extent the insects may be curbed by control of preseason conditions, such as clean culture to prevent the winter protection of the insect, by planting after the overwintered beetles have laid their eggs, which is May 1 to 15 for central Mississippi, and by rotation of crops.

White insects, called mealybugs, are occasionally found clustered more or less thickly on the nodules and roots of lespedeza, soy beans, red clover, white clover, and other legumes. The extent to which these insects interfere with nitrogen assimilation and the development of the plant is dependent largely on the number present on the root and the ability of the plant to withstand the attacks. Maximum damage may be expected when the moisture in the soil becomes insufficient for the needs of the plant. It has been reported by the Illinois Agricultural Experiment Station that under such conditions plants of red clover have been killed by mealybugs.

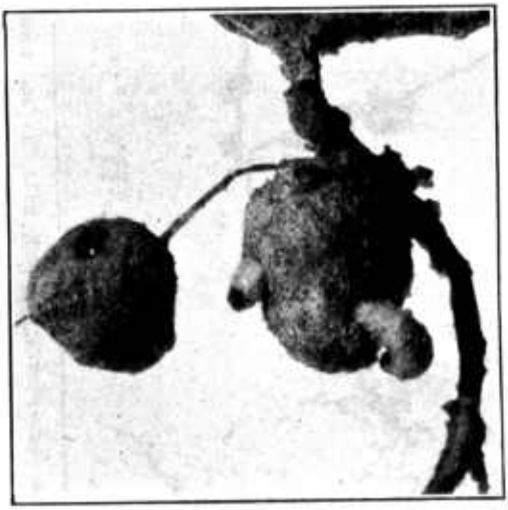

FIG. 12.—Nodules of cowpea injured by the larvæ of the bean leaf beetle. × 5

NITROGEN FIXATION BY LEGUMES

The nodules on all roots growing on an acre do not contain more than a few pounds of nitrogen, which can not account for the large quantities of nitrogen that are gathered by the legumes under suitable conditions. The continuous stream of starch and sugar passing from the leaves through the stems to the roots is partially used by the root-nodule bacteria with atmospheric nitrogen to form soluble nitrogenous compounds. These compounds are taken up by the plant and utilized in the building of its tissues. By this means legumes produce large quantities of organic material rich in nitrogen which is valued as human and animal food.

In order to get the greatest benefit, it is therefore necessary to select in each case those legumes which are best adapted to soil and climate. The possible gains in nitrogen and vegetable matter may be very small or very large, according to the conditions. Since soil and climate are highly variable factors, special tests must be made to find out in each case which legumes deserve preference. In addition, the economic side of the problem, of course, is also to be considered.

If legumes are grown as main crops, only those should be accepted as worth while which contain not less than 100 pounds of nitrogen per acre; 150 to 200 pounds are still more satisfactory, while with cover crops occupying the field for only a few months 60 to 80 pounds must be considered a good return. Whether or not all this nitrogen is actually taken from the air depends on the circumstances in each case. If there is much available nitrogen in the soil in the form of nitrate this may be taken up by the legumes and by the bacteria, too, and the latter may then assimilate but little from the air.

For all practical purposes it may be assumed that most of the nitrogen in a legume crop comes from the air, if a marked increase in the productivity of the soil is noticeable after the legumes have been harvested. Though a gain of 30 to 50 pounds per acre in field soils can not be ascertained with accuracy, even with very careful chemical analyses, this quantity of nitrogen in the soil has a marked effect on the succeeding crop. As a rule, poor soils are much improved if legumes are included in the crop rotation. Winter cover crops are very useful, crimson clover, hairy vetch, and sweet clover being especially suited for this purpose.

It is probable, although not definitely known, that in addition to the nitrogen fixation in their root nodules, legumes are of additional benefit by exerting a stimulating effect upon the nitrogen-fixing organisms which are living and acting in every soil. Their importance will be discussed later in this bulletin.

Average crops of cereals and potatoes remove from the soil approximately 30 to 50 pounds of nitrogen per acre in a year. If a legume is grown every fourth year as a main crop for forage, the manure produced from it contains one-half to three-fourths of what is needed to replace the nitrogen removed in the grain crops; and if a leguminous winter cover crop is grown, perhaps between wheat and corn, and fed as hay or silage, the manure obtained from it will completely cover the remaining deficit in nitrogen. Such a practice is the best assurance for maintaining, restoring, and increasing soil fertility.

BACTERIA ON AND AROUND THE ROOTS OF NONLEGUMES

Numerous bacteria of different kinds are present and active in every soil. Manuring, tillage, and plant growth influence their number as well as their activities. Certain nonlegumes, like potatoes, corn, and beets, have been found to exert a more favorable effect upon them than do small grains and timothy and other grasses.

Speculative investigators have tried repeatedly to adapt the root-nodule bacteria of leguminous plants to the roots of nonlegumes, and positive results have been announced. Careful tests, however, have not confirmed these claims, and it is highly improbable that the original nature of the higher plants as well as of the bacteria could be changed experimentally to such a degree that nonlegumes would be made to bear root nodules and harbor nitrogen-fixing bacteria in them, like the legumes. It has been mentioned that a few wild-growing nonlegumes are known to produce root nodules wherein perhaps nitrogen fixation takes place, but nothing of this kind has ever been observed with cultivated nonlegumes. Their bacteria are

only on the outside and in the neighborhood of the roots. If they exert a favorable action upon plant growth, this can be but indirect and is not very marked.

NITROGEN FIXATION BY BACTERIA AROUND THE ROOTS OF NON-LEGUMES

A considerable number of bacteria from different soils have been isolated which are able to fix elementary nitrogen, like the root-nodule bacteria, if they are abundantly fed in the laboratory with sugar or other suitable substances free from nitrogenous compounds. If they should find similar conditions in the soil their action would undoubtedly be very conspicuous. The root-nodule bacteria receive plenty of sugar from a leguminous plant. But the bacteria living in the soil have at their disposal only meager supplies of suitable food—some available humus, straw, and other crop residues, remnants of stable and green manures, and perhaps some sap exuding from healthy roots or from roots injured by worms, insects, etc.

Experiments made thus far have shown that, as a rule, not more than 10 to 20 pounds of nitrogen per acre may be added in a year by these free-living nitrogen-fixing bacteria to the store of organic nitrogen already present in the soil; occasionally 30 to 40 pounds have been recorded. But every soil, if it is not very poor, contains several thousand pounds of humus nitrogen per acre. This, as well as the nitrogen fixed by the soil organisms, must first be mineralized; that is, it must be converted into ammonia and nitrate before it will become accessible to the plant roots. This process takes time, and meanwhile there are many chances that these few pounds of nitrogen may be leached away together with many more pounds from the humus nitrogen, if the soil is not covered with plant growth and thus protected from the detrimental effects of heavy rains.

In permanent grassland, therefore, the nitrogen fixation around nonlegumes may often play a distinctly favorable part. In the old soils of Europe, where by very intensive cultivation and heavy applications of stable manure the most suitable conditions are maintained for a very active bacterial population and the utmost care is taken to reduce or to avoid losses in soil nitrogen by leaching or erosion, some beneficial effect is obtained from the bacterial activities in the soil. In American soils, however, because of the climatic extremes acting upon them, the losses in nitrogen will almost invariably surpass any gains. Furthermore, in America heavy applications of organic manures are rather the exception than the rule. They alone would furnish enough suitable food to the bacteria to increase their activity. If such a food supply is lacking, even specially selected bacteria that might have shown themselves very active in nitrogen fixation in laboratory experiments would be quite unable to demonstrate their capabilities in the soil. The situation is much the same as though a high-bred milk cow should be kept under very unsuitable conditions. In that case milk production would be rapidly reduced and soon stopped. With nitrogen-fixing bacteria no better result than reduction and failure can be expected under ordinary soil conditions.

This, of course, relates to those nitrogen-fixing bacteria that are present around the roots of the cultivated nonlegumes. It has been

mentioned that some wild-growing nonlegumes have root nodules filled with bacteria, much like the legumes. But these are not to be confounded with the generally grown crops of nonlegumes—cereals, potatoes, beets, flax, grasses, etc.

INOCULATION OF LEGUMES WITH NODULE BACTERIA

Long before anything was known about the nitrogen-fixing bacteria in the root nodules of leguminous plants, practical farmers, especially in the Netherlands, had seen great benefit from spreading small quantities of soil, taken from fields where legumes had been grown, over stretches of newly cultivated land, mainly sand and peat. After more definite knowledge of the bacterial process had

FIG. 13.—Sterilization of bottles for pure cultures of legume bacteria in the soil-bacteriology laboratory of the United States Department of Agriculture

been gained, the underlying reasons for the success of this method became evident. Since the transportation of soil is cumbersome and costly, and since the seeds of weeds, as well as plant parasites, may be carried from field to field in such soil transfers, bacteriologists soon began to cultivate the nodule bacteria in the laboratory and to use these cultures for legume inoculation. During the first few years the success obtained was very variable, because, as we now know, of imperfect knowledge about growing such cultures and keeping them active. Considerable progress has been made along these lines, and at the present time very efficient cultures may be obtained.

The distribution of cultures made by the United States Department of Agriculture is almost exclusively for educational purposes. (Fig. 13.) Only one culture is sent to an applicant, in order to give

him an opportunity to make a test. Larger supplies may be obtained from several of the State agricultural experiment stations at practically the cost of production. A list of State institutions and commercial firms that sell legume bacteria may be obtained from the Bureau of Plant Industry, United States Department of Agriculture.

Artificially prepared cultures are grown (1) in a nutrient solution, where they appear as a whitish turbidity and sediment; (2) on jellies, usually agar prepared from Asiatic seaweed, the surface of which is covered with the whitish slimy growth of bacteria; or (3) in sterilized soil. (Fig. 14.) Each of these methods of preparation has its advantages and disadvantages, and all three types of cultures are on the market. The directions which accompany the cultures must be followed very carefully in order to assure full success.

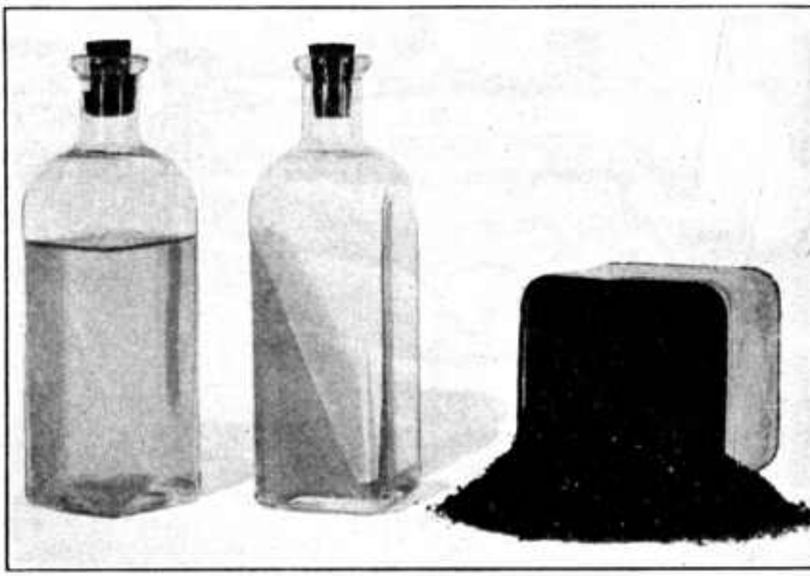

Fig. 14.—Cultures of root-nodule bacteria in liquid (at left), on agar (in center), and in soil (at right)

The great differences which may be caused by artificial inoculation are clearly shown in Figures 15 and 16. Crop increases amounting to several hundred per cent are by no means rare, especially in soils where these bacteria are absent before inoculation is attempted.

Even the most efficient cultures will be of little use if the conditions that are essential for obtaining a good stand of legumes are not fulfilled. Good tilth of the soil, clean, healthy seed, and a sufficient supply of lime, phosphate, and potash in the soil are the principal requirements. They have to be considered first, before the bacteria are asked to show what they can do. The weather during the growing season is likewise a factor. The nodule bacteria can not work satisfactorily unless they are amply supplied with sugar and starch by their host plants, which are unable to produce the necessary supply unless soil and climatic conditions are favorable.

If the seed contains disease-producing fungi or bacteria their activities may be stimulated by the moisture in the inoculation. Distributing the bacterial culture in dry soil and spreading this on the field may prevent this condition, but the use of clean, healthy seed, of course, is much more desirable.

INOCULATION OF NONLEGUMES WITH BENEFICIAL BACTERIA

There are two well-established means of inoculating soil for the growth of nonlegumes with various kinds of beneficial bacteria—the application of barnyard manure on the fields and of compost on grasslands and in the garden. If properly prepared, both materials are very rich in bacteria and carry at the same time organic and inorganic compounds that act as fertilizer. They add "life" to the soil, which means that many of the bacteria living in them multiply

FIG. 15.—Field of vetch, showing inoculated plants on the right and uninoculated plants on the left

rapidly in the soil and stimulate the chemical processes taking place in it. Not all of them are adapted to the conditions they find in the soil, and those bacteria will die. Some special tests have shown that the conditions may be such that even the manure bacteria remain practically without effect. Such exceptions, however, do not invalidate the general rule that regularly repeated applications of barnyard manure and compost are the best means of replenishing and rejuvenating the bacterial flora in soil growing nonlegumes.

All attempts at inoculation will remain unsuccessful if the conditions prevailing in the soil are not favorable for the growth of the bacteria contained in the cultures, but if they are favorable the bacteria almost without exception will be naturally present. Because these organisms are of a much less exclusive nature than the root-nodule bacteria of leguminous plants, they are to be found, at least in moderate quantities, in practically every soil. Careful tillage and

suitable crop rotations, together with the application of lime, organic manures, and fertilizers, if these are needed, will benefit the cultivated crops and the useful bacteria around their roots simultaneously.

REASONS FOR THE USE OF ARTIFICIAL CULTURES FOR LEGUME INOCULATION

To get the full benefit from their growth, legumes must either find the nodule bacteria in the soil or must be supplied with them. The

FIG. 16.—A well-inoculated peanut plant

plants will usually find bacteria of proper quality in sufficient quantity in the soil if the same kind of legume has been successfully grown continuously or in rotation on the same field, or legumes that harbor in their roots bacteria which belong to the same groups.

If soils are naturally rich in all different strains of nodule bacteria because their native flora contains many wild-growing legumes of various kinds, artificial inoculation is generally superfluous. On the other hand, if, for the first time, legumes are grown which have never been planted in that locality and whose bacteria are different from those occurring in the soil, inoculation is urgently needed. Soy

beans, hairy vetch, and alfalfa, for instance, have frequently failed in first trials merely because they were planted without being inoculated. But even where the same legumes have been grown before, inoculation may sometimes be advisable.

If soil is transferred from some other place as a means of inoculation, not only will the expense for labor be higher than if pure cultures were used, but the possible introduction of noxious weeds and plant parasites may ultimately cause serious financial losses. The diseases which have been reported as transferred in connection with soil inoculation are cowpea wilt and root-knot, and the pests are Johnson-grass seed, dodder seed, potato bugs, potato-tuber moths, and alfalfa weevils.

Lime should always be added and harrowed in sufficiently in advance of planting to allow for its physical and chemical action on the soil before sowing legume seed. The bacteria, on the other hand, should be present when the young roots are being formed; therefore it is best to inoculate the seed. On account of the caustic nature of hydrated lime there is great danger that many or most of the bacteria will be injured or killed if exposed to the direct action of such lime. Experiments have shown very conclusively that the manufacture and use of lime treated with legume bacteria are not to be encouraged.

OBJECTIONS TO THE USE OF ARTIFICIAL CULTURES FOR THE INOCULATION OF NONLEGUMES

The manner in which barnyard manure and compost are used in practical farming shows under what conditions the inoculation of the soil for nonlegumes may be successful. The bacteria contained therein are supplied from the start with relatively large quantities of suitable food, especially humus and humus-producing substances, which help to keep at least part of the added bacteria alive until they have adapted themselves to their new environment. In the same manner artificial cultures for the inoculation of nonlegumes would perhaps be successful if used in bulk, together with the necessary food supply.

In addition to their high prices, all these cultures are conspicuous by the exaggerated advertisements used to force their sale. Frequently it is claimed that "agriculture will be revolutionized" if the farmers will buy the special preparation. Such statements alone should suffice to raise grave doubts concerning the real value of the material. Agriculture least of all will be helped by any "revolution." The development of farming is a slow and steady process which proceeds from century to century, as economic conditions call for more intensive cultivation of the land. The high productivity of the old fields of Europe after many of them have been used for a thousand years or more shows very clearly that no "revolutionary" high-priced commercial cultures are needed for obtaining heavy crops of nonlegumes, but that thorough tillage of the soil, together with careful conservation and the use of organic manures as well as mineral fertilizers, will do all that can be expected.

Exact scientific investigations of the bacterial flora on and about the roots of nonleguminous plants are still much needed, and it is by no means unlikely that they will lead to further improvements in cultivating the soil. The results of such investigations should not

be confounded with the so-called discoveries offered to the farmer at exorbitant prices by high-sounding advertisements. Generally, the real value of these preparations is exactly the opposite of that heralded in the claims made for them by their manufacturers. The fact that a preparation is covered by a patent has no significance other than to indicate that the process of manufacture is different from that of anything else recorded in the files of the Patent Office. It has nothing whatever to do with the merits of the material.

Before spending any money for such preparations, information should be requested from a State agricultural experiment station or from the United States Department of Agriculture. Up to the present time, after examining more than 10 of these so-called biological fertilizers, the department has failed to find any which measured up to the claims made.

SUPERVISION OF THE DISTRIBUTION OF COMMERCIAL CULTURES

Unfortunately, not all manufacturers entering this field have the intention of establishing a permanent business. They recognize that, especially without the help of scientifically trained employees, some sorts of cultures can be produced very cheaply and with the help of glowing advertisements can be sold at high prices to credulous farmers. In a few months much money can be made in this way, as has been done in point of fact in connection with the manufacture of several of the so-called bacterial fertilizers for nonlegumes.

Accurate tests of such products require, in addition to laboratory experiments, carefully made inoculation experiments on the plants for which the preparations are sold. It always takes several months before final results are obtained, which delay often allows time for the unscrupulous dealer to sell much material.

The laws of Maryland and Wisconsin prescribe that persons or firms desiring to sell within their boundaries cultures of microorganisms for the stimulation of plant growth shall file a sworn statement of the composition of the material with certain State authorities who are vested with the power to refuse or cancel a permit when such material is sold under false and misleading claims. Violations of these laws may incur fine or imprisonment, or both. A somewhat similar law exists in New Jersey which, however, pertains to cultures of legume bacteria only.

Bacteria exist in the soil, for instance, which in pure culture show several features that are similar to those of the root-nodule bacteria. One of them (*Bacillus radiobacter*) is even to be found fairly regularly in nodules of soy beans, cowpeas, and related plants, and because its cultural characters are very much like those of the root-nodule bacteria of clover, alfalfa, vetch, and pea it has been mistaken repeatedly for the root-nodule organism of soy beans and cowpeas. Cultures of this organism used for inoculation prove, of course, entirely inefficient.

PRACTICAL TESTS OF THE VALUE OF INOCULATION

Whether the inoculation of legumes will be successful in a given case depends on several factors which can be determined only by

experiment. If a legume is planted which has not been grown before in that locality, inoculation as a rule is advisable, but good results may be obtained by the use of bacteria in other cases where the development of the plants is not very satisfactory and the nodules on the roots are not very numerous. First of all, it must be ascertained, of course, whether the soil conditions will favor a good growth of the legumes and of their bacteria. If the soil is acid it must be limed before planting; if it is low in potash and phosphate the proper quantity of fertilizers must be applied; if the tillage or drainage is poor it must be improved. After all has been done to obtain a good seed bed, clean, healthy seed must be available, and cultures for its inoculation must be obtained from a reliable source and handled properly.

It should never be overlooked that the bacteria contained in the cultures are minute living plants. Many of them will die when the cultures are kept for a long time, especially where the temperature is high and much moisture is lost by evaporation. Even the most careful supervision of the trade can not guarantee the good quality of every culture bought. Merely a few samples out of many thousands can be tested officially, and although these may be found very satisfactory it still depends on the handling of the cultures by the manufacturers and by the trade whether or not they will reach the farmer in good condition. Direct purchase from a manufacturing laboratory is preferable, since keeping it for a long time on the shelves of seed stores may easily prove harmful even to the best culture.

For a first trial, experimenting on a small scale is to be recommended. After the first test has given encouraging results, it should be repeated on a larger scale. But negative results obtained at first should by no means be accepted as final. Only repeated tests will lead to clear judgment in making a decision as to the value of inoculation.

Soil conditions are variable in every field, and an experiment should therefore be arranged so as to have inoculated and uninoculated plots of the legume under test on fairly similar representative sections of the field. Although it is almost self-evident, it is nevertheless too frequently overlooked that without the actual comparison of uninoculated and inoculated land no definite opinion can be formed.

If the difference between uninoculated and inoculated plants is not very marked, exact weighing of the crops grown on equal areas and careful examination of the roots are advised. With many legumes, especially when they are getting old, the nodules are easily stripped off the roots if these are pulled out of the ground. Therefore, the roots should be dug, but never pulled. On sandy soils it is sufficient to raise the plant with a spade and to shake the dirt carefully from the roots; in clayey and hard soils, it is better to raise the plant root in a ball of soil and to wash away the earth either in a bucket of water or by allowing a gentle stream to expose the root nodules.

The best time for making an inspection of the roots is during the period when the plant is in full vigor, preferably a short while before it comes into blossom. The number of days after planting in which nodules may be expected to become visible varies with

the conditions of soil and weather and with the kind of legume grown. Often the nodules begin to appear about two weeks after planting.

If a test has shown positive results and the whole area has been successfully inoculated, at the next planting it may be expected that no further inoculation will be needed for the special kind of legume used and for those legumes that harbor nodule bacteria belonging to the same group. On the other hand, it must be remembered that soil acidity, poor drainage, lack of organic matter, and excessive leaching, heating, or drying of the soil are more or less detrimental to the life of the bacteria in the soil. Under such conditions, therefore, repeated inoculations may prove useful.

With regard to the inoculation of nonlegumes, special care is necessary to avoid erroneous conclusions. The apparently successful tests frequently quoted in advertisements usually were made without precaution and often in complete disregard of the fundamental rules of careful experimenting. Usually no parallel tests have been made to overcome the lack of uniformity of soils, the crops have not been weighed with exactness, or excessive quantities of the so-called bacterial fertilizer have been applied in pot or plot tests of very small size where not the bacteria but the fertilizer ingredients have caused some crop increases, which, however, can not be obtained by the quantities economically applicable in the field. At present, the best advice which can be given to the farmer concerning all preparations offered for the inoculation of soils and nonlegumes is to abstain from experimenting with them and leave it to the agricultural experiment stations to find out whether they are of value. The pamphlets liberally distributed by the manufacturers of such preparations often contain quotations from well-known scientific books or from bulletins published by experiment stations. These quotations as a rule have nothing to do with the product offered for sale but are merely statements of the beneficial activities of soil bacteria, etc. They are not therefore in any sense justifications for the claims made and should not be accepted as indorsements of the products advertised.

DIRECTIONS FOR THE INOCULATION OF LEGUMINOUS PLANTS

Directions for the handling of inoculation cultures are always supplied with them, usually being printed upon the label or on the wrapper around the container. (Fig. 17.) Brief information regarding the use of field soil for inoculation has been given on previous pages. A few remarks may be added here, since inquiries concerning this matter are frequently received.

FIELD-SOIL INOCULATION

If a field has been planted to a certain leguminous crop and many nodules are found on the roots of this crop, the soil from this field may serve as a source of inoculation in other fields for the same crop or for crops harboring the same bacteria. Soils to be used for this purpose should be known to be free from objectionable weed seeds, plant diseases, and destructive insects. The use of large quantities of field soil for inoculation is advisable only when the source of supply is near the point of application.

The aim in collecting soil for this purpose is to get material in which the noduled roots have grown. If the inoculation has been recently introduced on the seed or has been sown in the rows with the seed, the bacteria will remain segregated around the seed or in the rows until scattered by some farming implement, such as a cultivator or harrow, or until they have slowly permeated the soil by natural growth.

Soil for transfer should be in a friable condition. The top crust should be scraped away and the soil for inoculation taken from the next 5 or 6 inches. Although the legume organisms will withstand the sun's rays to a considerable degree without being killed, excessive exposure is not advisable. Unless the soil is free from lumps and stones it should be sifted. A window screen may be used for sifting if nothing more convenient is available. Sifted soil (fig. 18) is especially necessary when it is to be sown through a drill.

FIG. 17.—Alfalfa culture in bottle, with label, as distributed by the United States Department of Agriculture

For the best results the rate of application should be liberal. It has been found that from 200 to 500 pounds of soil per acre will give adequate inoculation. The transferred soil may be spread by hand broadcasting or other means of distributing it on the surface; in either case it should be brushed or harrowed in at once. It is preferable from the labor standpoint to spread the inoculation simultaneously with the sowing of the seed. This may be accomplished successfully by distributing it through the fertilizer attachment of the drill.

Although the liberal use of soil is especially advised for soil inoculation, there are times when large quantities are not available or it is impracticable to obtain them. In such a case a smaller quantity may be adapted to a correspondingly smaller aereage, following the instructions for applying field soil, or it may be mixed with the seed

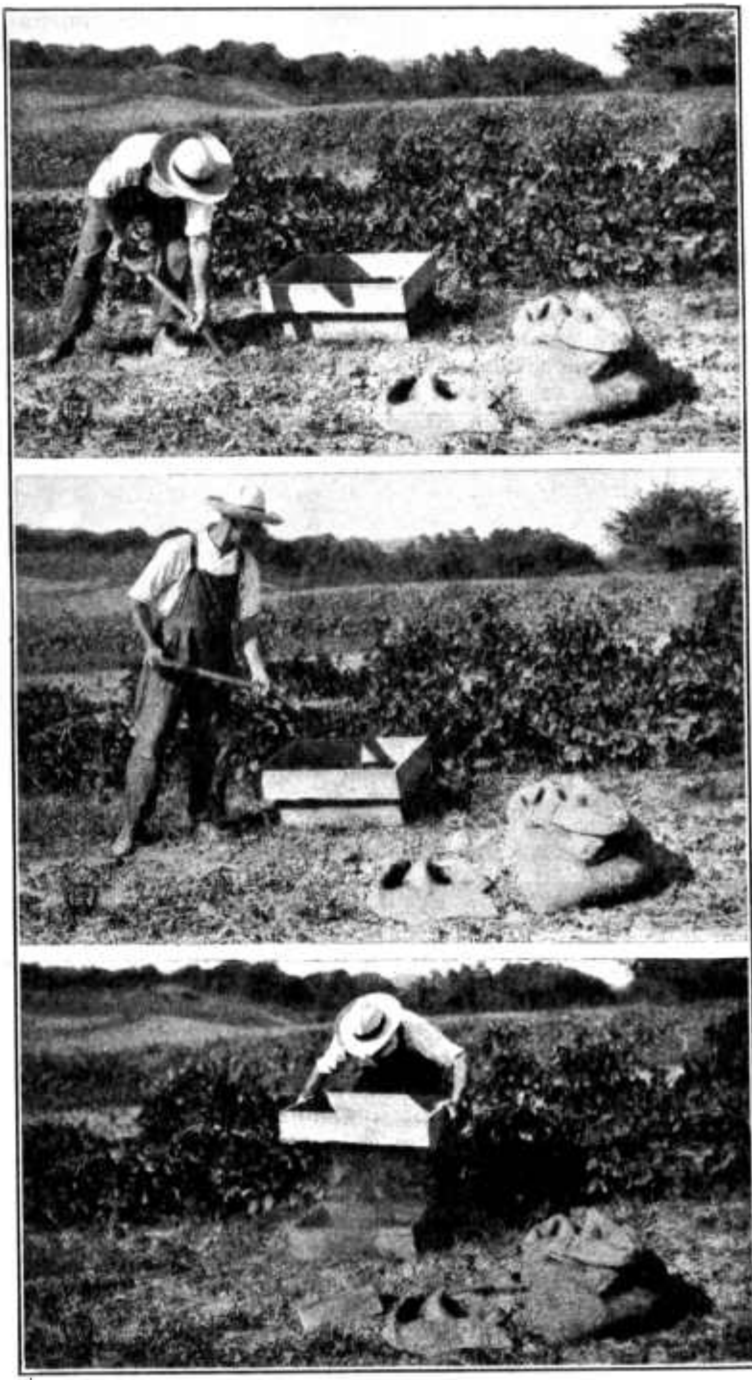

FIG. 18.—Preparing soil to be used for inoculation

and sown with it. Equal parts by weight of seed and soil have been found to give good results.

Another process probably as widely advocated as any is the "glue method" of inoculation. This method was devised at the Illinois Agricultural Experiment Station for the inoculation of large seeds with field soil. Two handfuls of ordinary cabinetmaker's glue are dissolved in a gallon of boiling water. After having cooled, the mixture is sprinkled on the seed at the rate of 1 pint per bushel. The seeds are stirred in order to spread the glue solution evenly over all of them. The inoculating soil, preferably dry for the purpose of quickly taking up the excess moisture of the glue solution on the seed, is added at the rate of 1 quart per bushel and thoroughly mixed with the seed. Part of the inoculating soil is thereby glued to the seed. The rate of seeding will be slower for seed so treated than for that untreated, and allowance should be made for this factor.

PURE-CULTURE INOCULATION

The pure cultures of legume bacteria are grown on specially prepared foods either in liquid or solid form. The latter is commonly agar, a jellylike substance made from seaweed. This material will not dissolve in water at ordinary temperatures, but it is possible to break it up into small particles. Sterilized soil mixtures are sometimes used in preparing pure cultures of legume bacteria, but in the process of manufacturing it often happens that the soil is not kept entirely free from foreign organisms.

Liquid cultures are ready for use, but soil and agar cultures must be shaken with water to obtain a suspension of the organisms. Liquid cultures and suspensions from the other cultures are applied usually to the seed, not to the soil. For small quantities of seed the organisms are generally sprinkled on the seed from the container, but for large quantities a sprinkling can is often used. Little batches of seed may be worked over by hand to effect the even distribution of the organisms (fig. 19), but with large quantities it is more satisfactory to shovel the seed about. Apparatus which may be used for the continuous inoculation of large quantities of seed may be found on the market.

If the seeds are not moistened excessively by the culture and are to be planted in a short time, it is fairly safe to put them in sacks immediately after inoculation. As a precaution, however, the freshly inoculated seed should be spread in the shade to become air dry. This may prevent the seed from deteriorating under excessively humid conditions.

Moist seed will feed through the drill more slowly than dry seed. Seed inoculated with pure cultures and then dried and kept for a long time will tend to lose its inoculation. It is not safe to keep seed much more than 10 days without reinoculating it. On this account it is advisable to apply the inoculation shortly before the time of planting.

USE OF MINERAL FERTILIZERS WITH INOCULATED SEED

The concentrated condition and the chemical reaction of mineral fertilizers commonly sold make it necessary to advise against the

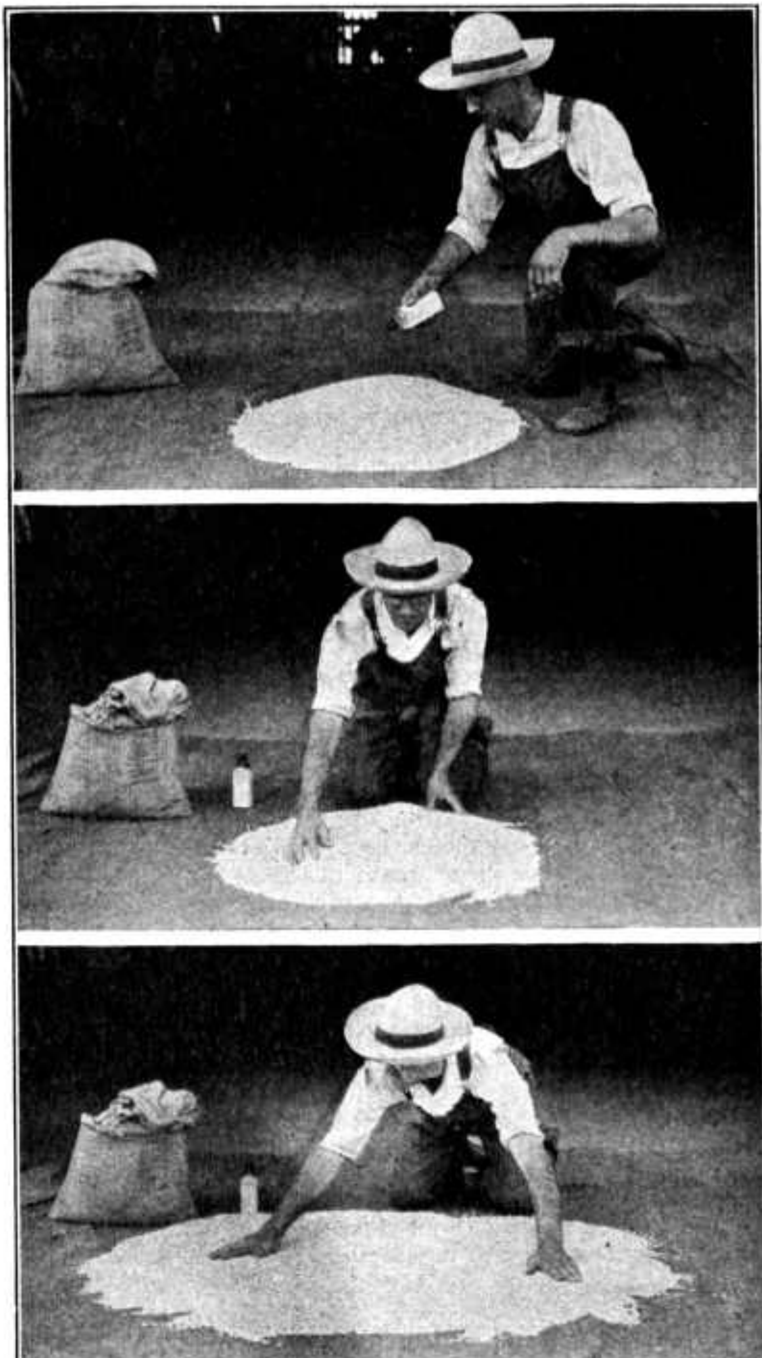

FIG. 19.—Inoculating seed with a liquid pure culture

direct mixing of inoculated seed with fertilizers. Tests made on inoculated seed directly mixed with various fertilizing materials showed that cyanamide of calcium, hydrated lime, and sulphur killed practically all the organisms on the seed and that acid phosphate and gypsum killed some of them, whereas bone meal, limestone, and a 4–12–4 fertilizer either had no detrimental effect or stimulated nodule production. On the other hand, soy beans sown dry with cyanamide, sulphur, and hydrated lime in soil known to contain the soy-bean organism were not appreciably affected in so far as nodule formation was concerned.

The results of the experiments alluded to and of other tests indicate that there will be no appreciable detrimental effect if the seed and the commonly used fertilizers are sown at the same time from their respective compartments of the drilling machine.

ORGANIZATION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

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